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Original Investigation

Developing a model of limited-access nicotine consumption in C57Bl/6J mice

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ABSTRACT

Although United States smoking rates have been on the decline over the past few decades, cigarette smoking still poses a critical health and economic threat. Very few treatment options for smoking exist, and many of them do not lead to long-term abstinence. Preclinical models are necessary for understanding the effects of nicotine and developing treatments. Current self-administration models of nicotine intake may require surgical procedures and often result in low levels of intake. Further, they do not lend themselves to investigating treatments. The current study sought to develop a limited-access model of nicotine intake using the Drinking-in-the-Dark paradigm, which results in high levels of binge-like ethanol consumption that can be pharmacologically manipulated. The present study found that mice will consume nicotine under a range of parameters. Intakes under the preferred condition of 0.14 mg/ml nicotine in 0.2% saccharin reached over 6 mg/kg in two hours and were reduced by an injection of R(+)-baclofen. Mecamylamine did not significantly affect nicotine consumption. As nicotine and ethanol are often co-abused, nicotine intake was also tested in the presence of ethanol. When presented in the same bottle, mice altered nicotine intake under various concentrations to maintain consistent levels of ethanol intake. When nicotine and ethanol were presented in separate bottles, mice greatly reduced their nicotine intake while maintaining ethanol intake. In conclusion, these studies characterize a novel model of limited-access nicotine intake that can be pharmacologically manipulated.

1. Introduction[1]

Although cigarette smoking is on the decline, the Centers for Disease Control (CDC) estimates that as of 2013, 17.8% of the US population was classified as current smokers. Further, cigarette smoking accounts for 1 out of 5 deaths, making it the leading cause of preventable disease. The economic cost of first- and secondhand smoke is vast. Almost $170 billion is spent yearly on medical care, whereas another $156 billion is lost in productivity due to smoking related illness (CDC). Approximately 68.8% of current smokers want to quit, yet quitting often takes multiple attempts and less than 10% of those who try to quit smoking successfully quit for at least 6 months (Messer et al., 2008).

Preclinical animal models, specifically those involving self-administration, can serve as a valuable tool to research the mechanisms of nicotine addiction as well as potential pharmacological treatments. Intravenous self-administration of nicotine mimics the rapid onset of nicotine inhalation in smokers (Lynch et al., 2010) and allows for quantification of how reinforcing the drug is. However, intravenous methods of self-administration require specific equipment, surgical catheter implantation, surgery recovery time, monitoring of catheter patency and pH of administered solutions, and often extensive operant training and food restriction to achieve escalation of responses and significant nicotine administration (Caille et al., 2012; Lynch et al., 2010).

Although non-operant oral self-administration studies do not offer the ability to quantify how reinforcing drugs of abuse are, they do allow for observation of self-administration and pharmacological manipulation of oral consumption. Few oral nicotine consumption studies have been conducted in mice. Of these, all but one has utilized 24 h, 2-bottle choice paradigms. Adriani et al. (2002) observed intakes around 0.8–1.2 mg/kg nicotine using a limited 2 h 2-bottle choice paradigm in male and female CD-1 mice. Although intakes were low, they produced pharmacologically relevant levels of cotinine, the main metabolite of nicotine. However, mice were also water deprived during the 22 h of the day when nicotine was not present. C57Bl/6J (B6) mice have routinely been shown to consume more nicotine than other strains in 24 h free-choice access paradigms. Relatively high levels of intake are consistent across nicotine concentration (Glatt et al., 2009; Klein et al., 2004; Locklear et al., 2012) and are not changed when a sweetener is introduced to the nicotine and non-nicotine solution or related to intake of sweetened solutions (Meliska et al., 1995; Robinson et al., 1996).

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[1] C57Bl/6J (B6), Centers for Disease Control (CDC), Drinking-in-the-Dark (DID), nicotinic acetylcholine receptors (nAChRs)

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These results may suggest that B6 mice are insensitive to the bitter taste of quinine relative to other inbred strains, particularly at higher concentrations. However, using a brief-access paradigm that reliably quantifies aversion to quinine, Glatt et al. (2009) demonstrated that B6 mice display similar oral aversion as other strains to increasing concentrations of nicotine.

The purpose of the current studies was to develop a limited-access model of rapid nicotine intake in B6 mice that was more suitable for testing potential pharmacotherapies than previous oral nicotine administration paradigms. We chose to model nicotine intake using the Drinking-in-the-Dark (DID) method developed for 20% ethanol. In the DID paradigm, B6 mice consume high binge-like levels of ethanol at rates that are much more rapid than during 24 h free-choice access paradigms (Matson and Grahame, 2011; Rhodes et al., 2005). We sought to characterize whether B6 mice would consume nicotine in a similar limited access, binge-like fashion, and under what specifications their intake was optimized by substituting ethanol for multiple concentrations of nicotine alone or nicotine with saccharin. In humans, smoking one cigarette over 5 min leads to absorption of approximately 0.3–2 mgs nicotine and smokers maintain cotinine levels of approximately 250–300 ng/ml (Hukkanen et al., 2005). It is important to note that, unlike ethanol, there is no clinical definition of what constitutes a nicotine "binge." Therefore, we refer to our model as a limited access, restricted period of rapid consumption without indicating clinical relevance.

Further, we sought to pharmacologically manipulate binge-like nicotine intake, as traditional ethanol DID offers a model that lends itself to prompt testing of pharmacological targets for alcohol and substance use disorders, unlike 24 h drinking models. To do so, we used the nAChR antagonist mecamylamine and the $GABA_B$ agonist R(+)-baclofen. Mecamylamine is often used to precipitate nicotine withdrawal whereas baclofen attenuates the negative effects of nicotine withdrawal (Varani et al., 2014). Both of these drugs reduce ethanol intake in the DID paradigm and baclofen has been shown to reduce smoking behavior in a clinical trial (Franklin et al., 2009; Hendrickson et al., 2009; Kasten et al., 2015; Leggio et al., 2015). Finally, we sought to characterize whether B6 mice would co-consume nicotine and alcohol, as is often the case in human addiction (National Institute on Alcohol Abuse and Alcoholism, 2007), and whether co-consumption could be pharmacologically manipulated.

2. Method

2.1. Animals

All animals were male B6 mice maintained on a 12:12 light cycle in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). In all studies mice were at least 60 days old at the beginning of testing. Animals had access to food and water at all times, apart from during nicotine and/or ethanol presentation when water was not available. All procedures were approved by the IUPUI School of Science Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (The National Academies Press, 2003).

2.2. Drugs

(−)Nicotine hemisulfate salt dissolved in H_2O (~40% w/v freebase) was purchased from Sigma Aldrich (St. Louis, MO). Nicotine was dissolved in quantities of 87.5, 175, and 262.5 μl per 500 ml of tap water to respective salt-form doses of 0.07, 0.14, and 0.21 mg/ml. Respective freebase nicotine doses for these concentrations were 0.028, 0.056, and 0.084 mg/ml. Saccharin was obtained from Sigma Aldrich (St. Louis, MO) and was dissolved in a 0.025% or 0.2% w/v solution using tap water. Ethanol (195 proof) was obtained from Pharmco, Inc. (Brookefield, CT), and solutions (10% v/v and 20% v/v) were made using tap water. R(+)-baclofen hydrochloride and mecamylamine hydrochloride were obtained from Sigma Aldrich (St. Louis, MO) and dissolved in 0.9% saline to concentrations of 0, 3, 6.5, and 10 mg for R(+)-baclofen studies and 0, 0.75, 1.5, and 3 mg for mecamylamine studies. Baclofen and mecamylamine solutions were delivered via intraperitoneal injections in a volume of 0.1 ml per 10 g of body weight. Experimental procedures including animal and drug usage are outlined in Table 1. All drug injections took place on day 5 immediately prior to fluid access.

2.3. Experiment 1: limited-access nicotine intake

Nicotine intake was characterized in 18 male B6 mice taken from our breeding colony maintained at IUPUI. These mice were first generation offspring of breeders purchased from Jackson Laboratories. For all studies, fluid was presented daily in 10 ml graduated tubes for two hours, three hours into the dark cycle. Fluid level was read to the nearest 0.05 ml at the beginning and end of the two hour access period. Empty "leak" cages were maintained for each fluid to determine fluid leakage over the 2 h access periods. Intake was calculated as fluid displacement for each individual mouse minus the fluid leak.

In Experiment 1, animals received 0.21 mg/ml nicotine in water alone, in water containing 0.025% saccharin, or in water containing 0.2% saccharin for five days of access. Following two weeks of water washout, the same animals were given five days of access to 0.14 mg/ml nicotine in 0, 0.025, or 0.2% saccharin. Groups were counterbalanced based on Saccharin Concentration during 0.21 mg/ml nicotine access. The 0.21 and 0.14 mg/ml doses fall within the high range of nicotine concentrations used in 24 h free-choice access studies. Further, nicotine-naïve B6 mice should have slight and no aversion to the 0.21 and 0.14 mg/ml concentrations, respectively (Glatt et al., 2009), allowing us to better understand how nicotine concentrations and saccharin edulcoration alters oral nicotine consumption.

During access to 0.14 mg/ml nicotine, mice were placed in an Opto M3 13″ × 9″ Mouse Cages locomotor activity system (Columbus Instruments, Columbus, OH). The animal's cage is placed directly into the monitor and locomotor scores are determined based on sensor beam breaks. Each cage monitor was started immediately following introduction of the reinforcer. Locomotor activity scores were collected in 5 minute bins during the two hours of reinforcer access and two hours post-reinforcer access.

2.4. Experiment 2: pharmacological manipulation of limited-access nicotine intake

A total of 64 male B6 mice were purchased from Jackson Laboratories. For pharmacological manipulation of nicotine intake, 0.14 mg/ml nicotine in 0.2% saccharin was used because it generated high levels of nicotine intake with low variability. Animals received 5 days of nicotine access. Thirty-two animals were administered 0, 3, 6.5, or 10 mg/kg of R(+)-baclofen. The remaining 32 animals were administered 0, 0.75, 1.5, or 3 mg/kg of mecamylamine. Doses were based on previous ethanol DID findings (Hendrickson et al., 2009; Kasten et al., 2015). On day 5, drug was administered immediately prior to bottles on and a fluid intake was monitored at bottles-on, hour 1, and hour 2 to observe time-specific effects on intake. Dose groups were counterbalanced based on day 4 nicotine intake and variance in intake across the 4 day acquisition phase.

2.5. Experiment 3: nicotine + ethanol limited-access intake

Because smoking and alcohol use disorders are highly comorbid, we also investigated co-consumption of nicotine and ethanol intake. Thirty-eight male mice were used to examine co-consumption of nicotine and ethanol. These mice had previously been used in a pilot study that was completed at least 3 weeks prior to the current study and had a 5 or 10 day history of 0.14 mg/ml nicotine in 0.2% saccharin or 0.2% saccharin